Capabilities for Planetary Protection: Safeguarding the crew and engineering systems for human missions to Mars

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Safeguarding Crew Health and Crew Systems

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CURRENT STATUS

- **Human exploration systems are habitats of multitude of poorly understood microbial communities, exposing crew to threat of infection or degradation of crew systems.**
- **NASA has developed microbial monitoring technologies to ensure compliance with planetary protection (PP) policies "to preserve our ability to study other worlds in their natural states; avoid contamination that would obscure our ability to find life —if it exists."**
- **Exploitation of already developed PP technologies is an effective way to establish and monitor the microbial risks associated with closed human habitation systems.**

DEVELOPED TECHNOLOGIES

- **ATP-based microbial detection system**
  - **Total biomass (dead and alive)**
    - **Rapid; ~5 min; TRL-4**
  - **Total viable population (alive only)**
    - **Rapid; ~30 min; TRL-4**
- **Gene-based microbial analysis system (TRL-4 to 6)**
  - **Conventional Cloning & Sequencing**
  - **DNA microarray:**
    - **Spores (Planetary Protection)**
    - **All members (Astrobiology, ISS-related)**
    - **Live microbes (Other national agencies)**
- **Applications in:**
    - **Aviation Security**
    - **Dept. Homeland Security**
    - **Biosensor (Crew Habitation)**
    - **Missions to Mars (Crew Health)**

ACHIEVEMENTS

Major Tasks (International Space Station):

- Microbial Observatory of ISS module (current NRA tasks)
- Characterization and monitoring of microbes in the ISS drinking water.
- Evidence of pathogenic microbes in the ISS drinking water: Reason for concern?
- Q-PCR based bioburden assessment of drinking water throughout treatment and delivery to the ISS.
- Molecular microbial community structure of REMS air system.

Major Tasks (Planetary Protection; Mars Program):

- Standardization of sample collection, processing, and analysis of biomolecules recovered from spacecraft and associated surfaces
- Genetic inventory of spacecraft and associated surfaces
- Molecular microbial monitoring of spacecraft surfaces of several NASA missions

Major Tasks (Commercial Aviation Program):

- Microbial bioburden and diversity of commercial airline cabin air during short- and long-duration of travel.
- Select human pathogenic viral burden assessment of commercial cabin air.

Major Achievements:

- Monitored several hundred drinking water samples retrieved from ISS and ITCS modules
- Coordinated a systematic approach with majority of the NASA microbiologists in microbial monitoring issues
- Transferred Mars Program developed technologies to Human Program

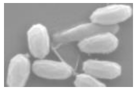
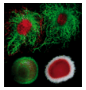
CAPABILITIES

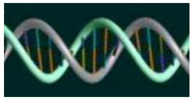
Technical capabilities:

- Rapid enumeration of microbes associated with low biomass surfaces and samples
- DNA microarray based phylogeny
- Next generation sequencing to assay deep microbial diversity
- Development of microbial monitoring systems to estimate viable bioburden present within fluid, surface, and air samples (ISS, planetary protection, ASTID, commercial aviation programs)
- Standardization and validation of end to end sample collection, process, and analyses

PARTNERS

- **NASA centers including JSC, KSC, Marshall, and ARC**
- **International collaboration: ESA, JAXA, and Kikkoman, Japan**
- **National Inst & Univ: LBNL, Stanford, and USC**

Developed state-of-the-art molecular technologies that enabled *bioburden measurement and mitigation plans for controlling microbial contamination* for robotic missions that can be utilized for human habitation missions.

Capabilities to be developed

- Technologies to estimate the viable microbial burden, as such measurements will alert crew members of any inherent threat of microbial illness and will forewarn of any bio-deterioration of their habitat.
- Microbial monitoring techniques will also be invaluable in assessing the degree of microbial dispersal into the pristine environment under investigation.
- An integrated, extensive sampling regime, an on-line/off-line monitoring system with an artificial intelligence–based feedback loop to interpret data, and plans for microbial mitigation and control to ensure sufficient longevity of flight hardware and an acceptable quality of life for the crew.
- An understanding of the microbial diversity present in system fluids and system surfaces to foster the development of methods to mitigate and control microorganisms for bio-corrosion, bio-fouling, and human pathogenesis.

ISS and Space Biology program funded work in microbial monitoring

- Present practices about microbial monitoring
- Consequences of microbial accumulation in closed environments
- NRC recommendations for future microbial monitoring and utilization
- Flight experiments:
  - ISS – Microbial Observatory:
    - Is the ISS environmental microbiome different from Earth cleanrooms?
  - ISS – Microbial Observatory of Pathogens:
    - Recent project with LLNL; Dr. Crystal Jaing – PI; JPL, JSC, and Ames are co-institutions

- **Present practices about microbial monitoring**
- **Consequences of microbial accumulation in closed environments**
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Microbial monitoring in ISS

- Culture-based
  - Contact slides for surface
  - Sartorius sampler for air
  - Filter methods for water
    - http://www.youtube.com/watch?v=wuIzl53Za80
- Samples return to Earth for processing and no in situ microbial monitoring system available (RAZOR, WetLab-2)
- No integrated instrumentation available that enable sampling to analysis
- No adequate data available about molecular microbial community of closed system
  - NASA Space Biology program just funded to collect samples for molecular analysis

Acceptability Limits

Air	
Total bacteria	1,000 CFU/m^3
Total fungi	100 CFU/m^3
Surfaces	
• Total bacteria	10,000 CFU/100 cm^2
• Total fungi	100 CFU/100 cm^2
Water	
• Heterotrophic plate count	50 CFU/ml
• Total coliform bacteria	Not detected in 100 ml

Courtesy of JSC

- Present practices about microbial monitoring
- **Consequences of microbial accumulation in closed environments**
- NRC recommendations for future microbial monitoring
- Flight experiments:
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Adverse Effects of Microorganisms

- Biodegradation
- Systems failure
- Food spoilage
- Release of volatiles

"…(fungi) feeding behind control panels, slowly digesting the ship's air conditioner, communications unit, and myriad other surfaces."

Gareth Cook, Boston Globe Staff (10-1-00)

Courtesy of JSC

Contamination Potential

Preflight contamination

Spacecraft are complex (cluttered)

Astronaut activities, such as eating and hygiene

Courtesy of JSC

Vehicle Design Controls

- HEPA air filters
- In-line water filters
- Contamination resistant surfaces
- Water biocides
- Water pasteurization systems
- Minimize condensation
- Contain trash and human waste

Courtesy of JSC

- Present practices about microbial monitoring
- Countermeasures to reduce contamination
- **NRC recommendations for future microbial monitoring**
- Flight experiments:
  - ISS – Microbial Observatory: Is the ISS environmental microbiome different from Earth
  - Recent project with LLNL (ISS – Microbial Observatory of Pathogens)

NRC Decadal Survey – ISS MO

- **In response to the National Research Council (NRC) Committee's Decadal Survey on Biological and Physical Sciences in Space, which reported that "*microbial species that are uncommon, or that have significantly increased or decreased in number, can be studied in a "microbial observatory" on the ISS.***

NRC. 2011. Committee for the Decadal Survey on Biological Physical Sciences in Space: Recapturing a Future for Space Exploration: Life and Physical Sciences Research for a New Era. The National Academies Press.

NRC recommendations to NASA

a) ***Capitalize on the technological maturity, low cost, and speed of genomic analyses and the rapid generation time of microbes to monitor the evolution of microbial genomic changes in response to the selective pressures present in the spaceflight environment***

b) ***Study changes in microbial populations from the skin and feces of the astronauts, plant and plant growth media, and environmental samples taken from surfaces and the atmosphere of the ISS***

c) ***Establish an experimental program targeted at understanding the influence of the spaceflight environment on defined microbial populations***

NRC. 2011. Committee for the Decadal Survey on Biological Physical Sciences in Space: Recapturing a Future for Space Exploration: Life and Physical Sciences Research for a New Era. The National Academies Press.

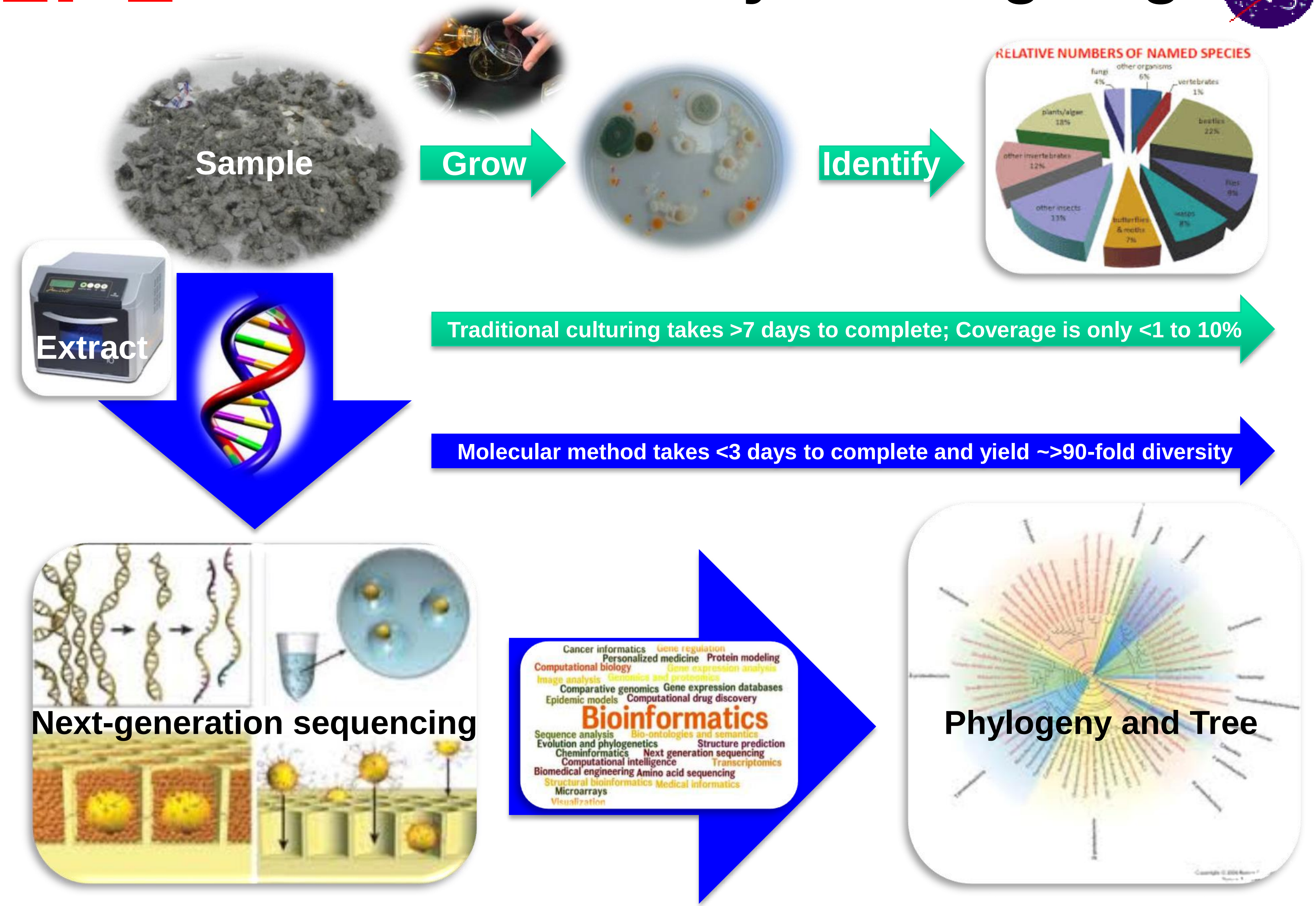
“Establish a “microbial observatory” program on the ISS”

– National Research Council

Flight experiments on ISS

- NASA – NRA (ISS microbial observatory)
  - Research opportunities in space biology – funded to JPL
  - JPL (PI) – JSC (Pierson; co-I) for US side of ISS
- NASA – NRA (Characterization of ISS HEPA filters)
  - Research opportunities in space biology – funded to JPL
  - JPL (PI) for HEPA filter analyses of ISS
- NASA – NRA (ISS microbial observatory for pathogens)
  - Research opportunities in space biology – funded to LLNL
  - LLNL (Crystal Jaing; PI); JPL (Venkat), JSC (Satish), and ARC (David Smith)

Microbial Inventory Cataloguing



Outline of the presentation

- Present practices about microbial monitoring
- Consequences of microbial accumulation in closed environments
- Microbes know where to hide
  - Some examples in Earth extreme environments
- NRC recommendations for future microbial monitoring
- **Flight experiments:**
  - **ISS – Microbial Observatory: Is the ISS environmental microbiome different from Earth cleanroom?**
  - Recent project with LLNL (ISS – Microbial Observatory of Pathogens)

Is the ISS Environmental Microbiome Different from the Earth Cleanrooms?

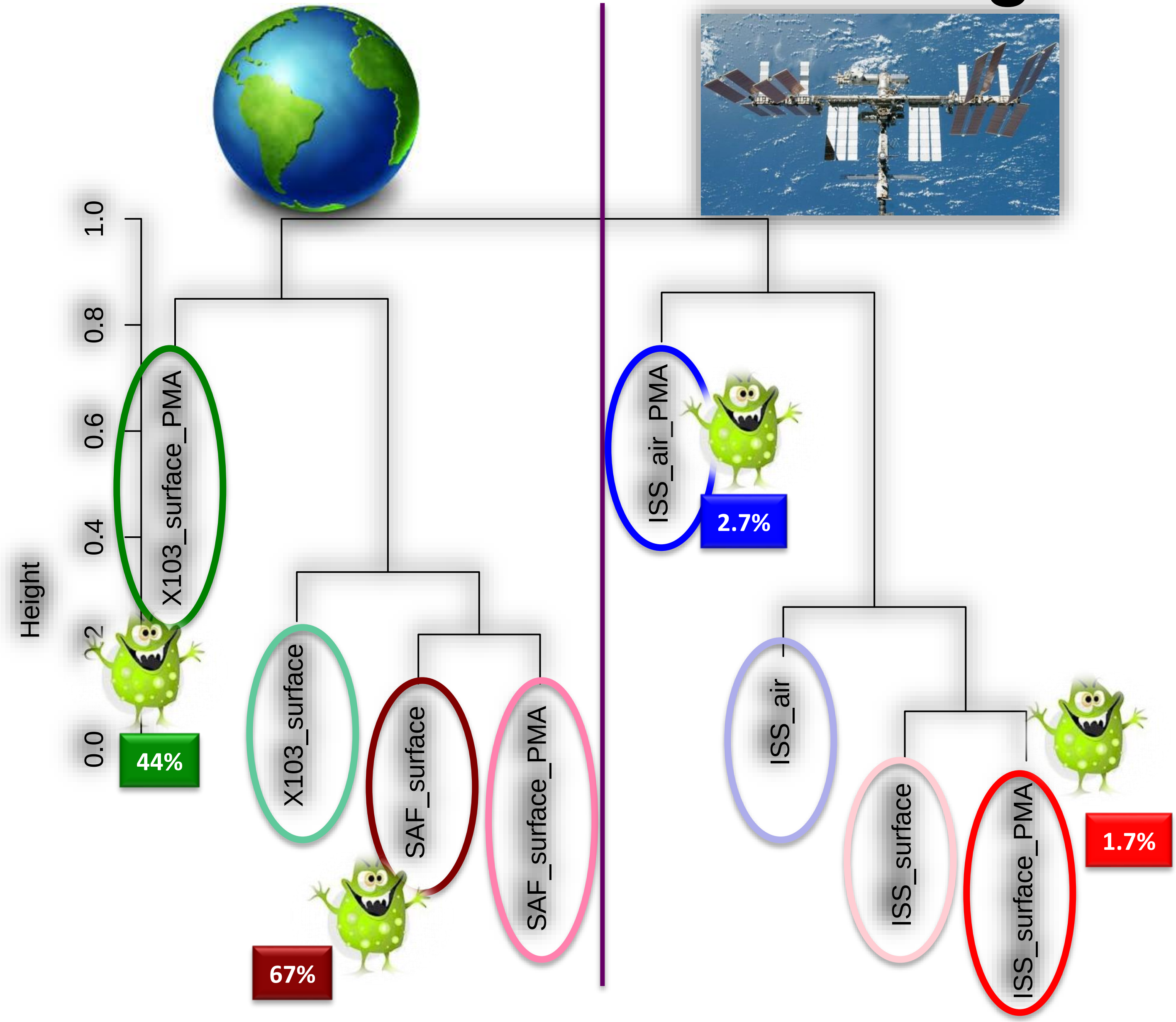
- Microbiome(s) of the surface sample collected via vacuum cleaner system was compared with the air sample collected via ISS HEPA filtration system.
- Vacuum cleaner system-based surface samples collected from two cleanroom facilities in Earth, that support a mission-critical spacecraft assembly at JPL and another one from an associated facility, were characterized for the indoor microbiome profiles and compared with the environmental microbiome of the ISS samples.
- Characterization of the bacterial, archaeal, and fungal diversities:
  - Traditional microbiology (cultivable bacteria and fungi)
  - State-of-the art molecular methods
    - ATP assay (measure viable microbial burden)
    - PMA-qPCR (quantify viable bacterial burden)
    - PMA-Next-gen sequencing (differentiate viable microbial diversity)

Characteristics of ISS and Earth analogue (spacecraft assembly facility) samples

Sample name	Location	Source	Type	Specifications	Duration	Model	Mission activities
ISS Filter Element	ISS Node 2	HEPA filter element	Air	HEPA rated, retains 99.97% particles >0.3 µm; 20-mesh inlet screen has 841 µm sieve openings	40 months	Part no. SV810010-1, Serial no. 0049; HEPA media supplied by Flanders Filters, Inc.; Nomex inlet screen	Returned aboard STS-134/ULF6 in May 2011
Debris from ISS Filter Element Inlet Screens	ISS	ISS Vacuum Cleaner bag dust	Surface	Vacuum bag retains particles >6 µm; HEPA rated filter retains particles >0.3 µm	1 day	International Space Station vacuum cleaner	Expedition 31; returned aboard Soyuz flight 29S in July 2012
JPL-SAF Debris	JPL – SAF Cleanroom Class 10K	Vacuum cleaner bag dust	Surface	HEPA rated filter retains 99.7% particles >0.3 µm	70 days	Nilfisk GM80, 81620000	Used for robotic missions
JPL-103 Debris	JPL – 103 Cleanroom Class 1K	Vacuum cleaner bag dust	Surface	HEPA rated filter retains 99.7% particles >0.3 µm	>180 days	Nilfisk GM80, 81620000	Sub-assembly of robotic missions

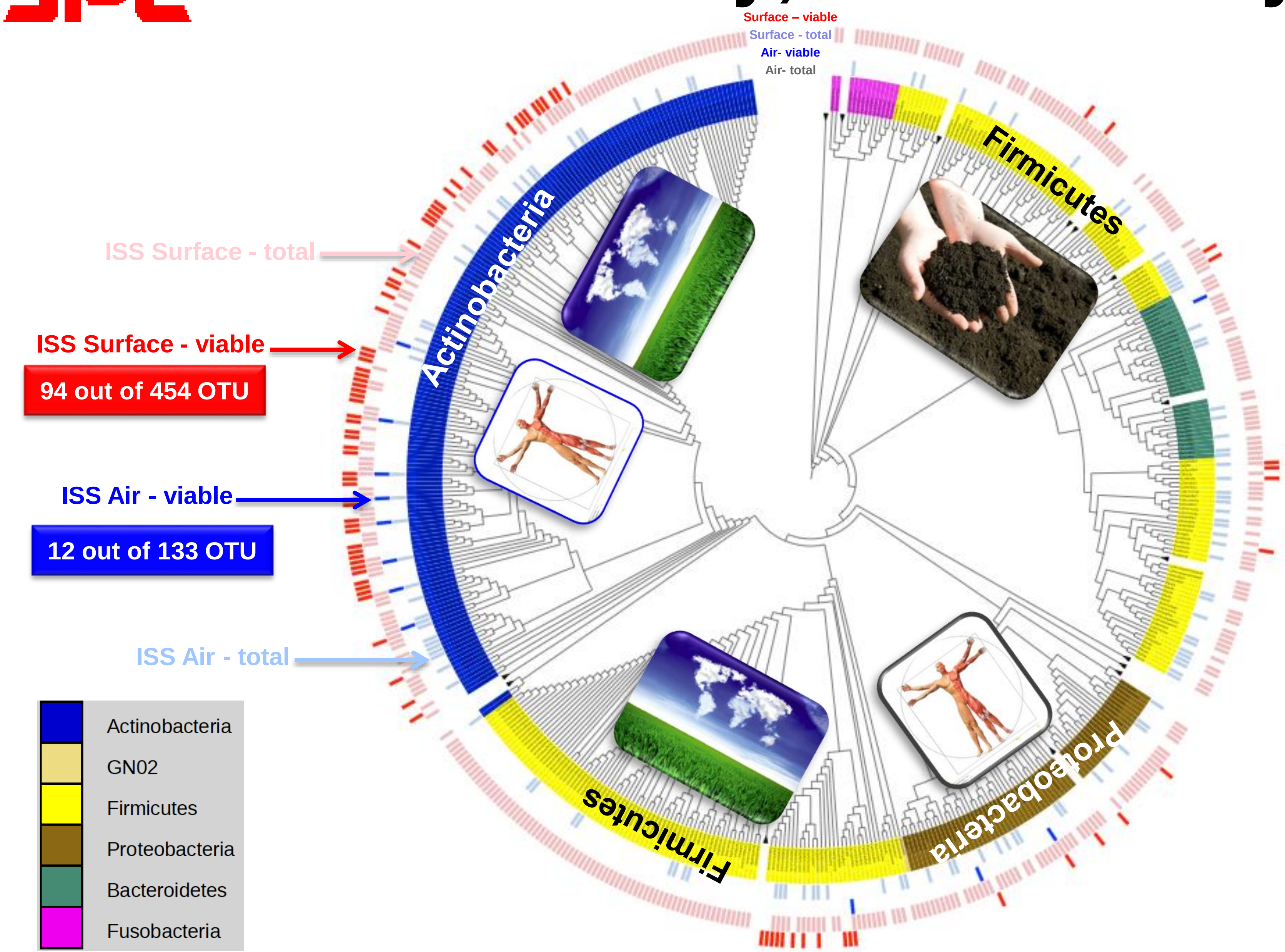
Is the ISS Environmental Microbiome Different from the Earth Cleanrooms?

Environmental clustering



Is the ISS Environmental Microbiome Different from the Earth Cleanrooms?

Bacterial Diversity; What are they?



Is the ISS Environmental Microbiome Different from the Earth Cleanrooms?

Dominant viable bacterial phyla

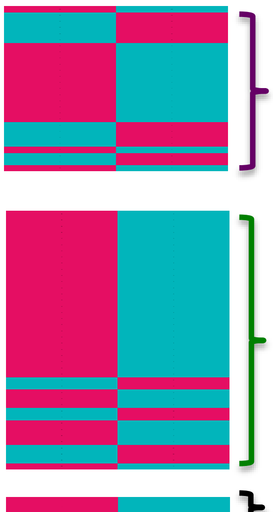
Phylum	ISS HEPA Viable	ISS Debris Viable	JPL-SAF Debris Viable	JPL-103 Debris Viable
Total # of sequences	587,569	1,116,419	1,472,777	397,607
% sequences that belong to three dominant phyla	99.65	98.26	81.84	90.46
Actinobacteria				
% of sequences	95.28	66.54	25.25	21.46
# of genera	55	38	116	71
# of dominant genera (>100 sequences)	7	16	76	24
Firmicutes				
% of sequences	3.97	28.48	11.05	0.98
# of genera	67	31	150	53
# of dominant genera (>100 sequences)	17	18	69	7
Proteobacteria				
% of sequences	0.41	3.24	45.55	68.02
# of genera	65	30	191	92
# of dominant genera (>100 sequences)	7	10	104	29

Is the ISS Environmental Microbiome Different from the Earth Cleanrooms?

ISS – MO and ISS – MOP projects

- ISS – MO is already funded and first collection of samples was performed and returned for analysis
  - Project outline
  - Preliminary results will be discussed
- ISS – MOP is just awarded and in Flight Definition Phase. On April 1, 2015, Dr. Crystal Jaing, will be having dialogue with NASA Space Biology program managers at Ames.

Are these viable microbial population (PMA-treated) "problematic?"



Bacteria	Agent	Disease
Actinobacteria	*Propionibacterium acnes* 	Acne disease
Firmicutes	*Staphylococcus epidermidis* 	Skin rashes
Proteobacteria	*Methylobacterium* sp. 	No disease

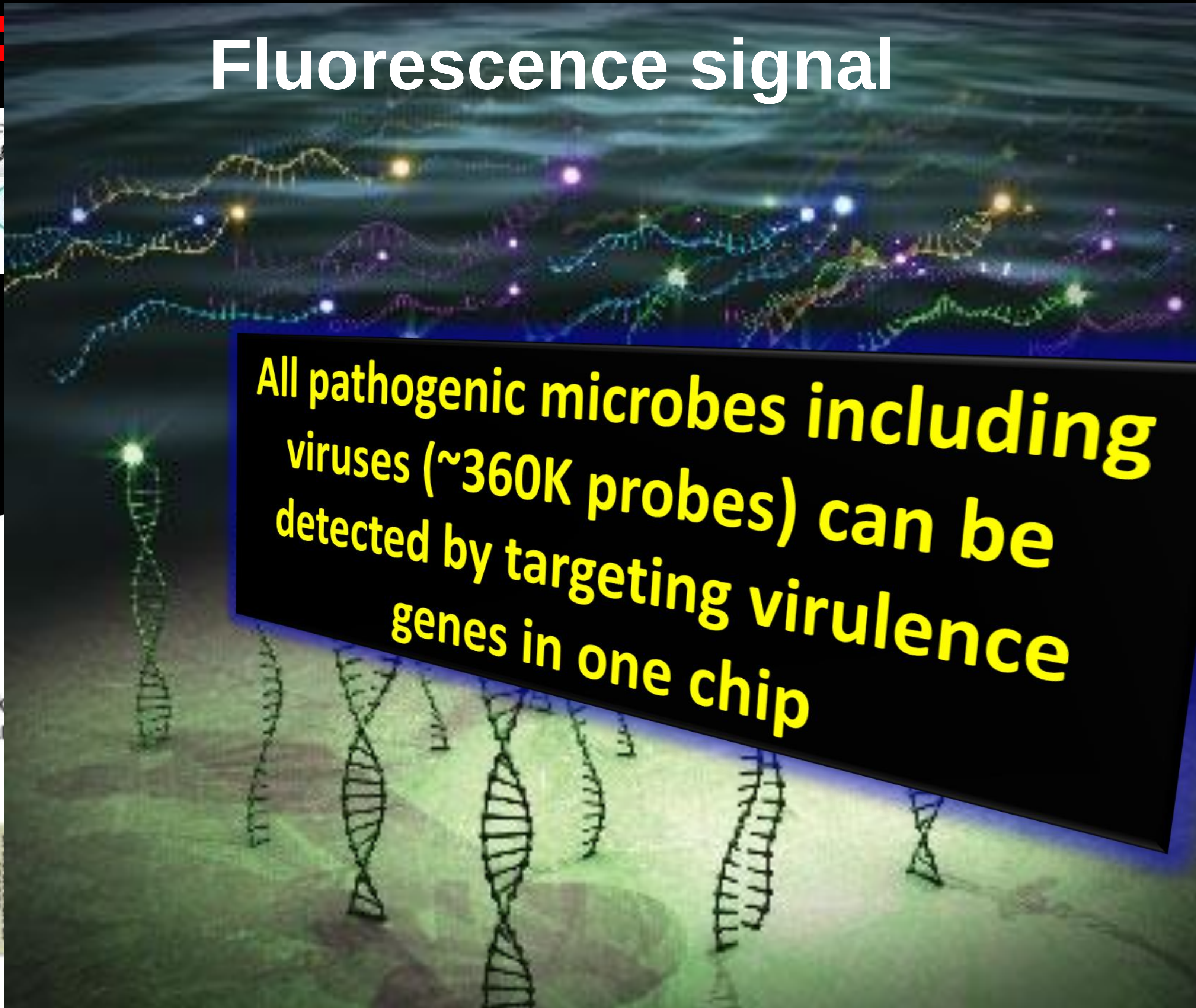
Not to panic now!!!

- Even though these pathogens are alive, the virulence needs to be tested and for that we need live cultures.
- It is basically not possible to culture these pathogens in ISS and there is no capability to bring them live to Earth.
- Solution: Measure the virulence of predominant microbes using microarray platform.

Heat map of Operational Taxonomic Unit (OTU) across ISS debris sample treated with and without Propidium MonoAzide (PMA). OTU with more than ten sequences in at least one sample were considered to construct this heat map. *Green* represents low OTU abundance while *red* represents high abundance.

Venkateswaran et al. 2014. International Space Station environmental microbiome — microbial inventories of ISS filter debris. Appl. Microbiol. Biotechnol.: 1-14.

Fluorescence signal



All pathogenic microbes including viruses (~360K probes) can be detected by targeting virulence genes in one chip

Hybridize sample on microarray

The Team

$$$: NASA Space Biology Program

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